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A comprehensive study on the inclusion mechanism of benzophenone into supramolecular nanoassemblies prepared using two water-soluble associative polymers

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Special Chapter dedicated to the memory of dr. Michel Ollivon

Abstract In this study, the entrapment of benzophenone (BZ) into supramolecular nanoassemblies prepared by mixing two water-soluble associative polymers (i.e. polymerized β-CD (pβ–CD) and dextran grafted with lauryl-side chains (MD)) has been investigated by using isothermal titration microcalorimetry (ITC) and molecular modeling. ITC experiments have been performed at various temperatures (4 °C (277 K), 25 °C (298 K), and 37 °C (310 K)) to evaluate the interaction of BZ with pβ–CD in comparison with β-CD. The inclusion complexation for both β-CD/BZ and pβ–CD/BZ interactions was entropy-driven ($|\Delta H| < |T\Delta S|$) when the temperature of the experiment was low (4 °C) and enthalpy-driven ($|\Delta H| > |T\Delta S|$) with minor entropic contribution when the temperature was increased (25 and 37 °C). Using all the thermodynamic data obtained for β-CD/BZ and pβ–CD/BZ interactions when the temperature of the experiment was varied, the $\Delta H = f(T\Delta S)$ plot was perfectly linear, which reflected an enthalpy–entropy compensation process. Finally, the combination of ITC data with molecular modeling provided consistent information in regard to the location of MD side chains and BZ inside the cyclodextrin cavity, as well as concerning the stability of the nanoassemblies loaded with BZ.

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Introduction

Over the past few years, benzophenone (BZ), was widely used as sun screen in the cosmetic field. However, as most of the UV absorbers used in sunscreen cosmetics, BZ is poorly soluble in water (about 0.25 mM at 25 °C) [1]. Since solubility limitations represent, for many compounds, a serious concern when foreseeing efficient formulations, one strategy for increasing their apparent water solubility is the complexation with cyclodextrins (CDs) [2, 3]. Shaped as a hollow truncated cone, CDs are cyclic oligosaccharides of D-(+) glucopyranose units all in chair conformation linked by α-(1,4) glucosidic bonds. This particular structure enables CDs to accommodate into their cavity a wide variety of hydrophobic molecules, modifying, thereby, their physico-chemical properties such as the improvement of the apparent solubility, and the protection against photolysis. However, in the case of BZ, previous research study has demonstrated that the use of native β-CD resulted in the formation of insoluble BZ/pβ–CD complexes when β-CD concentration was increased above 0.75 g L^{-1} (0.66 mM) [1]. Besides these limitations due to the solubility of the β-CD, generally, CDs do not represent carrier systems able to control the release of the entrapped drug.

Therefore, there is an urgent need to develop new systems to overcome these limitations. In this context, our group has recently proposed a solvent-free procedure to obtain colloidal nanoassemblies of 100–200 nm containing CDs [4]. These nanoassemblies were formed spontaneously in aqueous medium upon the association of two

water-soluble polymers: a hydrophobically modified dextran by grafting alkyl-side chains (MD) and a poly-β-cyclodextrin polymer (pβ–CD) (Fig. 1). The stability of the system is assured by the formation of inclusion complexes between the alkyl chains (C12) of the MD and the β-CD cavities [5]. Interestingly, in this system, some β-CD cavities remain available to include hydrophobic molecules such as BZ. Recently, the entrapment of BZ into self-assembling cyclodextrin-based nanogels has been successfully achieved [1]. Furthermore, in the presence of pβ−CD, a continuous linear increase of BZ solubility was observed when the pβ–CD concentration was increased. The phase solubility diagram was a typical A_L-type [6] indicating the formation of soluble BZ/pβ–CD complexes for the CD polymer. Furthermore, the highly water-soluble pβ–CD allowed a spectacular increase of the hydrophobic molecules' solubility. For example, a polymer concentration of 75 g L^{-1} (concentration of β-CD cavities = 46 mM) could enhance BZ solubility by 63-fold, while a β-CD concentration of 15 g L^{-1} (concentration of β-CD cavities = 0.13 mM), near to saturation, allowed only a 1.5-fold increase of BZ apparent solubility [1].

From a practical point of view, the pβ–CD polymer was pre-loaded with BZ, previously to its contact with MD (Fig. 1). The entrapment efficiency of BZ into the

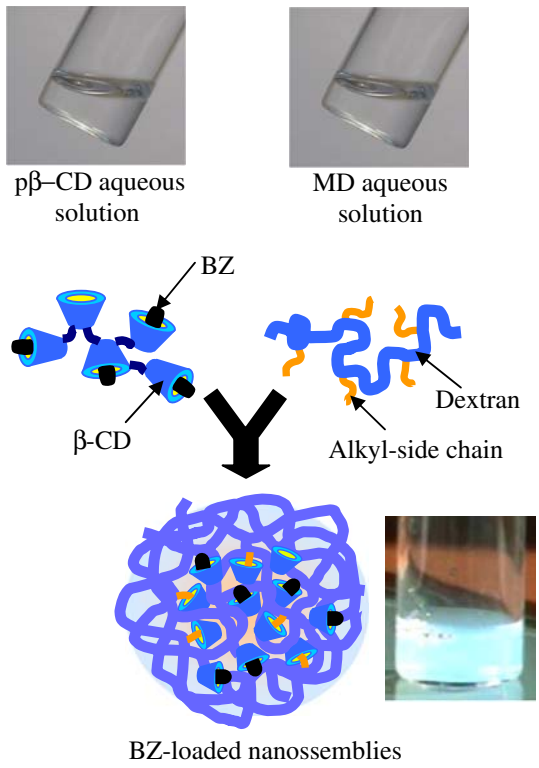

Fig. 1 Schematic representation of the formation of the supramolecular nanoassemblies loaded with BZ by mixing the two water-soluble associative polymers MD and pβ–CD

nanoassemblies was about 20% [1]. Thus, the presence of BZ inside CD cavities neither disturbed the nanoassembly formation, nor destabilized the system. Since BZ molecules are theoretically able to compete with the alkyl-side chains of MD, the question of the stability of the system, even after the inclusion of BZ, remained to be explained. In this context, we propose here to investigate many possible interactions occurring in the system: the inclusion of MD and BZ into CD cavities of the pβ–CD polymer and the interaction of MD with BZ.

Furthermore, the cosmetic formulations need to be stable over a wide range of temperatures [7], and the association constants are known to depend on this factor [2]. Therefore, the study of the variation of the association constants and thermodynamic parameters, when the temperature of the experiment is changed, is of utmost importance to better understand the complexation mechanism of BZ with pβ–CD. In addition, this allows also to predict the encapsulation yield and the release profile of BZ from pβ–CD when the temperature is varied. Since such investigation has never been reported yet, it was interesting to explore the effect of the variation of temperature (4, 25, and 37 °C) on the inclusion of BZ into pβ–CD.

Among the various techniques available for the characterization of host–guest interactions, ITC is the only method which provides a direct and accurate measurement of association constant and thermodynamic parameters of the interaction under the variation of environmental conditions [3, 5, 8]. This technique leads to the direct determination of the association constant (K) and the enthalpy of the interaction (ΔH), from which the free energy (ΔG), the entropy (ΔS), and heat capacity changes (ΔC_p) on the interaction of MD and BZ with pβ–CD can be calculated. In this present study, ITC will be used in combination with molecular modeling to reach information about the mechanism of the association of BZ into pβ–CD and about the stability and cohesion of the BZ-loaded nanoassemblies.

Materials

β-cyclodextrin (β-CD) was purchased from Roquette (France). Benzophenone (BZ) was supplied from Sigma (France). The purity was >97%. Water was purified by reverse osmosis (Milli-Q, Millipore®, USA).

pβ–CD was prepared by crosslinking β-CD under strongly alkaline conditions with epichlorohydrin (EP) [9]. In brief, 100 g of anhydrous β-CD was dissolved in 160 mL of NaOH 33% w/w solution under mechanical stirring overnight. Then, 81.52 g of EP (molar ratio (β-CD/EP) = 10) was rapidly added to the solution heated to 30 °C. In order to obtain a high molecular weight polymer, the reaction was stopped in the vicinity of the gelation

point by addition of acetone. The obtained aqueous phase was heated to 50 °C overnight, neutralized with 6 N HCl and ultrafiltered using membranes with a cut-off of 100,000 g/mol. The β-CD polymer was finally recovered by freeze-drying. The β-CD content, as determined by ^{1}H NMR spectroscopy, was 70% w/w. The average molar mass of pβ–CD polymer was 7×10^5 g/mol, as determined by size exclusion chromatography using pullulan standards.

Dextran bearing hydrophobic lauryl-side chains (MD) was synthesized as previously described [10, 11]. In brief, 4 g of dextran (40,000 g/mol) solubilized in 100 mL of dimethyl formamide containing 1 g of lithium chloride, were reacted for 3 h at 80 °C with 0.43 mL of lauroyl chloride and 0.031 mL of pyridine. The obtained MD was isolated by precipitation in isopropyl alcohol. It was further solubilized in distilled water, purified by dialysis for 48 h and finally freeze-dried. The substitution yield of MD was 5% of glucose units, as determined using ^{1}H NMR spectroscopy.

Methods

Preparation of solutions for ITC experiments

The amount of water in each sample used for ITC experiments (β-CD, pβ–CD, and MD) was accurately determined by weighing the samples before and after drying under vacuum at 105 °C during 24 h.

- β-CD and pβ–CD solutions were prepared by dissolving the corresponding weight of β-CD and pβ–CD powder into MilliQ® water.
- MD solutions were prepared by dissolving the corresponding weight of MD powder into MilliQ® water. In order to allow complete MD solubilization, solutions were magnetically stirred overnight.
- Solutions of BZ were prepared as follows. An excess of commercial BZ (25 mg) was placed in a glass vial together with 50 mL of MilliQ® water under magnetic stirring overnight at 25 °C. A suspension was obtained and filtered through a 0.22-µm membrane filter (Millex, SLAP 0225, Millipore, France). After appropriate dilution with MilliQ® water, BZ concentration in the filtrate was measured spectrophotometrically at 260 nm (Perkin Elmer UV/VIS spectrophotometer, Germany).

Isothermal titration microcalorimetry (ITC) experiments

The isothermal calorimeter instrument (MicroCal Inc., USA) was used to determine stoichiometry (N), binding constant (K), and thermodynamic parameters for the interactions—β-CD/BZ, pβ–CD/BZ, pβ–CD/MD and MD/BZ. Three protocols were achieved with the aim to investigate these interactions:

- **Protocol 1** In order to study the interaction of BZ with β- and pβ–CD, a syringe filled with 283 µL of β-CD (10 mM) or pβ–CD aqueous solution (concentration of β-CD cavities = 10 mM), was used to titrate an aqueous solution of BZ contained into the sample cell. In order to study the effect of temperature on the interaction, temperature of the experiment was varied: 4 °C (277 K), 25 °C (298 K), and 37 °C (310 K). A background titration, consisting in injecting the same titrant solution (β-CD or pβ–CD) in solely MilliQ® water placed in the sample cell, was subtracted from each experimental titration to account for dilution effects.
- **Protocol 2** In order to study the interaction between MD and pβ–CD, aliquots of 10 µL of titrant consisting of MD (concentration of alkyl chains = 7.50 mM) placed in a syringe were used to titrate pβ–CD solution (concentration of β-CD cavities = 1 mM) placed in the sample cell. The temperature of the experiment was fixed at 25 °C (298 K). A background titration, consisting in injecting the same MD solution in solely MilliQ® water placed in the sample cell, was subtracted from each experimental titration.
- **Protocol 3** In order to study the interaction of MD with BZ, aliquots of 10 µL of titrant consisting of MD (concentration of alkyl chains = 7.50 mM) placed in a syringe were used to titrate BZ solution (0.25 mM) placed in the sample cell. The temperature of the experiment was fixed at 25 °C (298 K). A background titration, consisting in injecting the same MD solution in solely MilliQ® water placed in the sample cell, was subtracted from each experimental titration. For protocols 1 and 3, the BZ solution was placed into the sample cell due to its low solubility in aqueous media.

For all three protocols, the titrant solution was delivered over 25 s, and the corresponding heat flow was recorded as a function of time. Intervals between injections were 600 s to allow complete equilibration and agitation speed was 394 rpm.

Data consisting in series of heat flows were collected automatically and when appropriate, the interaction process between the two species has been analyzed by means of either one-site or two-site binding model proposed in the Windows-based Origin 7 software package supplied by MicroCal. Based on the concentrations of the titrant and the sample, the software used a nonlinear least-squares algorithm (minimization of Chi2) to fit the series of heat flows (enthalpograms) to an equilibrium binding equation, providing best fit values of the stoichiometry (N), the

association constant (K), and the change in enthalpy (ΔH). From these results, the free energy (ΔG) and the entropy (ΔS) were deducted according to the equations: $\Delta G = -RT \ln K = \Delta H - T\Delta S$.

Molecular modeling

Molecular modeling was employed to simulate the interaction between MD and pβ–CD in the presence of BZ and water molecules, using both molecular mechanics (MM2, MM3) and semi-quantum mechanics (AM1 and PM3). First, two linear chains of pβ–CD (8 units) and MD (16 units) were inserted within a cylinder containing 720 water molecules. The system was energy minimized and subjected to molecular dynamics (300 K, step 1 fs, heating/cooling rate 1.000 kcal/atom/ps) for a trajectory of 95 ps. The nanoassembly was further modified by adding a BZ molecule in the fourth β-CD cavity, and the system was subjected to molecular dynamics for an additional 25 ps (same conditions).

Results and discussion

It has been demonstrated that nanoassemblies are formed instantaneously when MD and pβ–CD in aqueous solution are mixed together because the hydrophobic CD cavities of the pβ–CD can serve as host for the pendant alkyl chains grafted on the hydrophobized dextran [4]. Interestingly, numerous empty CD units remained accessible for the inclusion of hydrophobic molecules such as BZ [1]. The loading of the BZ into the nanoassemblies was performed by simply mixing pβ–CD aqueous solutions containing the BZ molecule with MD solution.

The cohesion of these stable structures is based upon a "lock-and-key" mechanism and one can wonder about the reasons of the stability of the system even after the inclusion of BZ which is expected to compete with the alkyl-side chains of MD. In this context, the knowledge of the binding constants and the thermodynamic parameters of the different interactions which can occur in the system are of central importance for understanding the phenomena of the molecular interaction of BZ when loaded into nanoassemblies. With this aim, a set of experiments were achieved by studying the following interaction β-CD/BZ, pβ–CD/BZ, pβ–CD/MD, and MD/BZ.

Interaction of BZ with MD

It has been demonstrated in previous studies that the amount of solubilized BZ increased in a linear manner by increasing the MD concentration [1]. This suggested the occurrence of interactions between BZ and MD making necessary the confirmation of such interactions by using ITC. The ITC results of the MD/BZ interaction are presented on Fig. 2a, showing that exothermic heats were released when BZ solution was titrated by MD. These exothermic heats were significant compared to the small endothermic heats observed upon the dilution of MD into water (Fig. 2c). The BZ solubilization may result from interactions with the hydrophobic cores (alkyl-side chains of MD) of the polymeric micelle-like aggregates formed by MD. However, the interaction of MD with BZ is too weak to allow the determination of the association constant and thermodynamic parameters.

In the light of these results, in the case of nanoassemblies, BZ might be located inside CDs, but possibly also inside hydrophobic microdomains of the MD formed when alkyl-side chains are in excess.

Thermodynamic data analysis

Then, we tested following interactions: BZ/β-CD, BZ/pβ–CD and MD/pβ–CD. The best fit of the binding curve (right panels of Figs. 2, 3) was obtained when using the standard one-site binding model (1:1) leading to the direct determination of the stoichiometry of the interaction (N 1:1), binding constant (K), and the enthalpy (ΔH) released upon the interaction between BZ and cyclodextrins (Fig. 3) and between MD and pβ–CD (Fig. 2).

The formation of a 1:1 complex between BZ and pβ–CD is characterized by an association constant (K) expressed by the following equation:

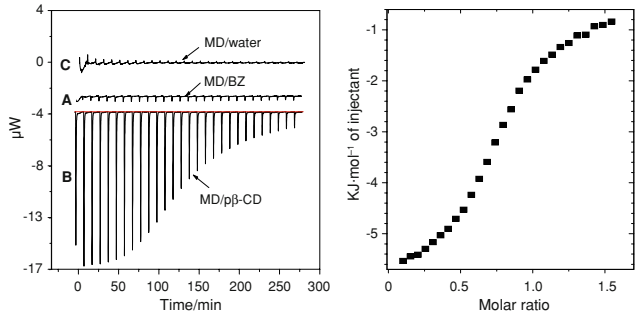

Fig. 2 Left panel shows exothermic heat flows which are released upon successive injection of 10 μL aliquots of MD (7.50 mM) into BZ solution (0.25 mM) (*curve A*) according to protocol 3 and pβ–CD solution (1 mM) contained into the sample cell according to protocol 2 (*curve B*). Control consists on the injection of MD (7.50 mM) in MilliQ® water (*C*). The right panel shows integrated heat data as a function of the molar ratio of MD and pβ–CD, giving a differential binding curve, which was fit to a standard single-site binding model yielding the following parameters $N = 0.95$, $K = 12{,}600\ M^{-1}$, and $\Delta H = -5.66\ kJ\ mol^{-1}$. Calculated parameters were $T\Delta S = 17.84\ kJ\ mol^{-1}$ and $\Delta G = -23.5\ kJ\ mol^{-1}$. Temperature of the experiment was fixed at 25 °C (238 K)

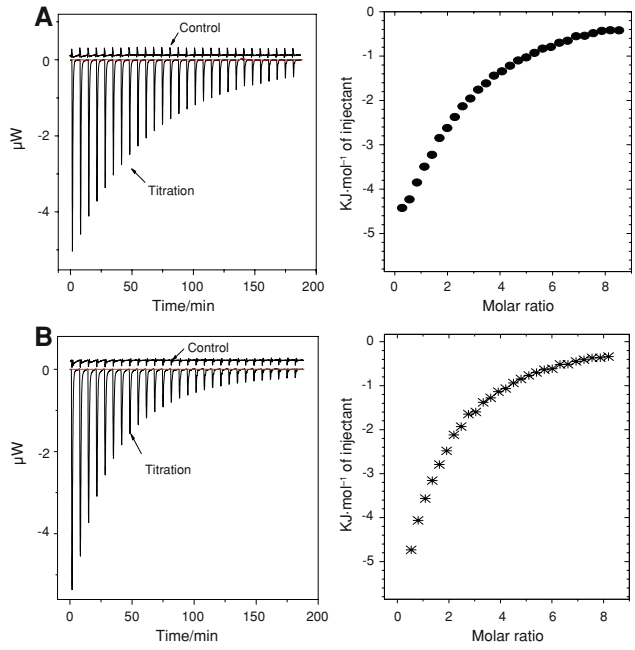

Fig. 3 Typical ITC data corresponding to the binding interaction of BZ (0.25 mM) with β-CD (10 mM) (*filled circle*) and pβ–CD (10 mM) (*asterisk*) in MilliQ® water at 4 °C (277 K). Left panels show exothermic heat flows which are released upon successive injection of 10 μL aliquots of β-CD (**A**) and pβ–CD (**B**) into BZ solution. Right panels show integrated heat data, giving a differential binding curve which was fit to a standard single-site binding model yielding the following parameters: N, K, ΔH, and ΔS (Table 1). Controls consist of the injection of β-CD (**A**) and pβ–CD (**B**) into MilliQ® water

$$K = \frac{[\mathrm{BZ} \cdot \mathrm{CD}]}{[\mathrm{BZ}][\mathrm{CD}]} \quad (1)$$

while the association constant corresponding to the formation of a 1:1 complex between MD and pβ–CD is expressed by Eq. 2:

$$K = \frac{[\text{MD-alkyl} \cdot \mathrm{CD}]}{[\text{MD-alkyl}][\mathrm{CD}]} \quad (2)$$

[BZ], [CD], [MD-alkyl], [BZ·CD] and [MD-alkyl·CD] are the concentrations of BZ solution, cyclodextrin, alkyl chains of modified dextran, and the inclusion complexes, respectively.

So far, the most probable mode of binding involves the interaction of the less polar region of the guest molecule with the CD cavity while the more polar groups of the guest are exposed to the bulk solvent outside the wider opening of the CD cavity. Many events, including desolvation of water molecules bound to the guest molecule and/or to the CD and formation of weak bonds (hydrogen bonds, hydrophobic interactions), electrostatic bonds, between the guest molecule and the CD, result in balanced ΔH and ΔS variations. Van der Waals forces and hydrophobic interactions related to the size/shape matching between guest molecule and CD's cavity are those among the several possible weak noncovalent interactions which provide the most essential contributions upon the complexation of organic guests with CDs. The study of ΔH and ΔS leads to the differentiation between these two types of forces. The interaction is dominated by van der Waals forces when the process is enthalpy driven with minor favourable or unfavourable entropies of interaction $|\Delta H| > |T\Delta S|$ [12]. However, hydrophobic interactions between two apolar molecules at room temperature have been known as entropy-driven processes, where the entropy of interaction is large and positive while the enthalpy of the process is small ($|\Delta H| < |T\Delta S|$) [13].

For the interaction of MD with pβ–CD, the analysis of thermodynamic data when the temperature of the experiment was fixed at 25 °C led to the conclusion that the association process was predominantly driven by entropy and moderately by enthalpy ($\Delta H < 0$; $T\Delta S > 0$; and $|\Delta H| < |T\Delta S|$). Large positive ΔS changes usually arise from the significantly important translational and conformational freedoms of host and guest upon complexation [14, 15]. From size-fit concept, there is clear evidence that the cavity size of β-CD was too large to provide a significant contribution due to van der Waals-type interactions. As a result, the flexibility of the supramolecular complex formed was high, resulting in a large gain in ΔS [5].

Effect of temperature on the interaction of BZ with β-CD and pβ–CD

Let us examine now the effect of temperature on the association constant of the interaction of BZ with both β-CD and pβ–CD. Table 1 shows that much higher affinities and much stronger interactions were obtained when the temperature of the experiment was decreased (37, 25, and 4 °C).

With regard to other thermodynamic parameters, when the temperature of the experiment was fixed at 25 °C, the association process was predominantly enthalpy driven ($|\Delta H| > |T\Delta S|$) (Table 1). When the temperature of the interaction was high (37 °C), the process was also dominated by enthalpy. However, when the temperature of the experiment was low (4 °C), the interaction of BZ with β-CD and pβ–CD was entropy driven ($|\Delta H| < |T\Delta S|$). As explained in the previous section, the entropic gain usually arises from the important translational and conformational freedoms upon the interaction of BZ with CD cavity of the polymer. Furthermore, the desolvation on BZ inclusion and the induced dehydration from peripheral hydroxyl groups of CD cavity in the polymer when the temperature of the experiment is lowered appear to be responsible for the entropic gain. [12, 16]. From these findings, it could be concluded that the interactions between BZ and

Table 1 Stability constants (K), and thermodynamic parameters for inclusion complex formation of BZ with both β-CD and pβ–CD for temperatures of 4 °C (277 K), 25 °C (298 K), and 37 °C (310 K)

Sample	T/°C	K/M^{-1}	ΔH/kJ mol^{-1}	$T\Delta S$[b]/kJ mol^{-1}	ΔG[b]/kJ mol^{-1}
β-CD	4 (277 K)	3,770 ± 16[a]	−9.20 ± 0.92	9.76 ± 1.00	−18.96 ± 0.08
	25 (298 K)	2,680 ± 14	−16.77 ± 0.86	2.77 ± 0.94	−19.55 ± 0.08
	37 (310 K)	1,560 ± 10	−18.94 ± 0.75	0.01 ± 0.83	−18.95 ± 0.08
pβ–CD	4 (277 K)	3,480 ± 15	−7.16 ± 0.88	11.61 ± 0.96	−18.78 ± 0.08
	25 (298 K)	2,710 ± 13	−12.60 ± 0.74	6.97 ± 0.82	−19.58 ± 0.08
	37 (310 K)	1,880 ± 9	−17.59 ± 0.52	1.84 ± 0.61	−19.43 ± 0.09

[a] Mean SD ($n = 3$)

[b] $\Delta G = -RT\ln K = \Delta H - T\Delta S$

cyclodextrins represented the net effect of solvation changes (hydrophobic hydration) and van der Waals interactions with the predominance of each type of forces when the temperature of the experiment was changed.

The formation of BZ/β-CD and BZ/pβ–CD complexes was spontaneous in all the cases, as evidenced by the negative value of the Gibbs free energy ΔG (Table 1). It is worth noting that the ΔG variations when the temperature of the experiment was changed, were regarded as barely significant (Table 1). This can be attributed to the compensation of the enthalpic changes by the negative $T\Delta S$ variations. Indeed, a good straight line ($R^2 = 0.997$) could be obtained when plotting ΔH versus $T\Delta S$ for the different reactions of complexation of BZ with β-CD and pβ–CD. This confirmed the existence of an enthalpy–entropy compensation effect (Fig. 4). The slope very close to unity ($\alpha = 1.065$) indicates that the inclusion complexation caused substantial conformational changes, probably involving the reorganization of the original hydrogen bond network when the temperature of the experiment was changed [17].

Enthalpy–entropy compensation due to variation in temperature can be shown to be a consequence of thermal heat capacity (ΔC_p) effects as it has been demonstrated and well explained in many articles [18, 19]. The negative value of the ΔC_p (−0.30 kJ mol^{-1} K^{-1}) calculated from the slope of the curve $\Delta H = f(T)$ is attributed to water reorganization upon transference of BZ from bulk water to CD cavity [16, 20–22].

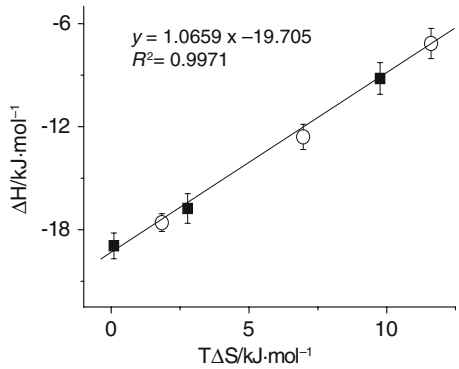

Fig. 4 Enthalpy–entropy compensation plot corresponding to inclusion complex formation of BZ (0.25 mM) with β-CD (*filled square*) (10 mM), and pβ–CD (*open circle*) (10 mM). Temperature of the experiment was changed (4 °C (277 K), 25 °C (298 K), and 37 °C (310 K)). See Table 1 for the original data

Stability and cohesion of the nanoassemblies loaded with BZ

Molecular modeling studies brought several elements of information which complement well the ITC studies. Molecular modeling was employed to simulate the interaction between MD and pβ–CD in the presence of BZ and water molecules. In our simulations, when a segment of pβ–CD (containing two linear chains of 8 units, see Experimental Section) was put into contact with MD (featured by a 16-unit chain), MD tightly interlaced around pβ–CD, which tended to adopt a bent helical structure (Fig. 5). Thus, molecular modeling confirmed the stability of the system (via molecular dynamics) and the presence of hydrophobic interactions which led to the insertion of the MD-alkyl chains inside the β-CD cavities could thus be evidenced. The BZ molecule stayed tethered inside a β-CD cavity, whereas the two alkyl chains spontaneously inserted inside the nearest β-CD cavities. Consequently, pre-insertion of BZ in a CD did not disturb the interaction between MD and pβ–CD.

We have previously shown that MD established an extensive network of hydrogen bonds with pβ–CD and that the established network was stable (a trajectory of 400 ps at 300 K was acquired) [4]. On the other hand, it has been previously shown by molecular modeling that β-CD could easily accommodate up to 12 hydrogen-bonded water molecules within its so-called hydrophobic core [23]. In our simulations, molecular dynamics clearly demonstrated the displacement of water molecules from β-CD cavities as a result of alkyl chain inclusion. The resulting "free" water molecules are no longer at a distance compatible with the establishment of hydrogen bonds. As their number

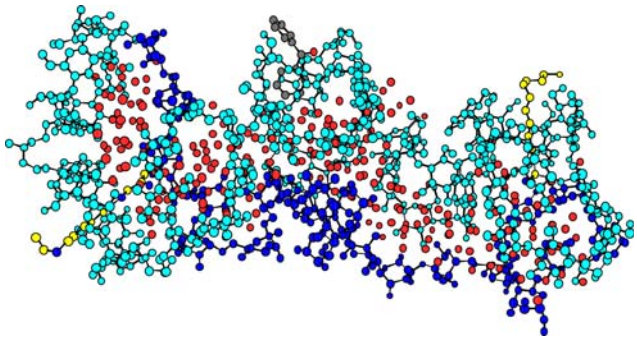

Fig. 5 Molecular modeling of the supramolecular nanoassemblies using both molecular mechanics (MM2, MM3) and semi-quantum mechanics (AM1 and PM3). Two linear chains of p-βCD (8 units, *turquoise*) and MD (16 units, the dextran chain is *blue*, C12 side chains are *yellow*) were inserted within a cylinder containing 720 water molecules (*red*). The system was energy minimized and subjected to molecular dynamics (300 K, step 1 fs, heating/cooling rate 1.000 kcal/atom/ps) for a trajectory of 95 ps. The nanoassembly was further modified by adding a BZ molecule in the fourth β-CD cavity, and the system was subjected to molecular dynamics for an additional 25 ps (same conditions)

increases, it can be inferred that polymer organization in nanoassemblies was essentially entropy driven.

At 25 °C, the association constant between BZ and β-CD was found around 2,680 M^{-1} (Table 1). This is in a 10-fold order of magnitude higher than the previously published association constants between alkyl chains and β-CD [24]. Thus, an alkyl chain would insert inside a free vicinal β-CD rather than displace a pre-included BZ from a β-CD. This accounts for the previously reported molecular modeling studies, showing that the presence of a pre-included BZ molecule did not interfere with the mechanism of formation of the nanoassemblies.

Thus, the cohesion of the nanoassemblies is presumably due to the establishment of numerous weak interactions between alkyl chains on MD and β-CD from pβ–CD, acting as physical cross-links between the chains of the two associative polymers. It is worth noting that the association constant between alkyl chains on MD and pβ–CD is high (12,600 M^{-1}) in comparison with the values of association constant between alkyl chains and β-CD reported in the literature [24]. The association of the two polymers, MD and pβ–CD, can be related to the "zip mechanism". Indeed, when one akyl chain of the MD is included into β-CD cavity, the other alkyl chains on MD become in proximity with other available CD cages in pβ–CD [5].

Conclusions

The combination of ITC results and molecular modeling, led to reach consistent information about the mechanism of the entrapment of BZ into auto-associative nanoassemblies obtained by mixing pβ–CD and MD. Furthermore, this study provided a more comprehensive understanding of the nature of noncovalent interactions operating between BZ and β-CD cavity as a function of the temperature and proved the occurrence of an interaction between BZ and MD.

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